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ATTORNEY'S DOCKET NUMBER
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U.S. APPLICATION NO. (If Known, see 37 CFR 1.5)
10/009383

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/12257	4 MAY 2000	4 MAY 1999

TITLE OF INVENTION
PROTEINS EXPRESSED BY MYCOBACTERIUM TUBERCULOSIS AND NOT BY BCG AND THEIR USE AS DIAGNOSTIC REAGENTS AND VACCINES

APPLICANT(S) FOR DO/EO/US
Maria Laura Gennaro

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (Unsigned)
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern other documents or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    - ☒ PTO Form 1449
    - ☒ International Search Report
    - ☒ 4 References
    - ☐
    - ☐

CERTIFICATE OF MAILING BY EXPRESS MAIL Express Mail Label No EF 045320336 US

I hereby certify under 37 CFR §1 10 that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C 20231.

November 2, 2001	Valente Figueroa	Valentin Figueroa
Date of Deposit	Signature	Typed Name of Person Signing

U.S. APPLICATION NO. (IF KNOWN) 10/009383	INTERNATIONAL APPLICATION NO. PCT/US00/12257	ATTORNEY'S DOCKET NUMBER 07763-043001

17. ☒ The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)- (5)):

	CALCULATIONS PTO USE ONLY	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... **$1040**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$100.00	
Surcharge of **$130** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$0.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	34 - 20 =	14	x **$18**	$252.00	
Independent Claims	2 - 3 =	0	x **$84**	$0.00	
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ **$280**	$0.00	
			TOTAL OF ABOVE CALCULATIONS =	$252.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$176.00	
			SUBTOTAL =	$176.00	
Processing fee of **$130** for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f))				$0.00	
			TOTAL NATIONAL FEE =	$176.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$0.00	
			TOTAL FEES ENCLOSED =	$176.00	
			Amount to be refunded:	$	
			Charged:	$	

a. ☒ A check in the amount of $176.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 06-1050 in the amount of $0.00 to cover the above fees. A duplicate copy of this sheet is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1050. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

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PROTEINS EXPRESSED BY MYCOBACTERIUM TUBERCULOSIS AND NOT BY BCG AND THEIR USE AS DIAGNOSTIC REAGENTS AND VACCINES

The invention is in the field of tuberculosis and, specifically, reagents useful for generating immune responses to *Mycobacterium tuberculosis* and for diagnosing infection and disease in a subject that has been exposed to *M. tuberculosis*.

Background of the Invention

Tuberculosis infection continues to be a world-wide health problem. This situation has recently been greatly exacerbated by the emergence of multi-drug resistant strains of *M. tuberculosis* and the international AIDS epidemic. It has thus become increasingly important that effective vaccines against and reliable diagnostic reagents for *M. tuberculosis* be produced.

U.S. application no. 08/796,792 is incorporated herein by reference in it entirety.

Summary of the Invention

The invention is based on the inventor's discovery that a polypeptide encoded by an open reading frame (ORF) in the genome of *M. tuberculosis* that is absent from the genome of the Bacille Calmette Guerin (BCG) strain of *M. bovis* elicited a delayed-type hypersensitivity response in animals infected with *M. tuberculosis* but not in animals sensitized with BCG. Thus proteins encoded by ORFs present in the genome of *M. tuberculosis* but absent from the genome of BCG represent reagents that are useful in discriminating between *M. tuberculosis* and BCG and, in particular, for diagnostic methods (e.g., skin tests and *in vitro* assays for *M. tuberculosis*-specific antibodies and lymphocyte responsiveness) which

discriminate between exposure of a subject to *M. tuberculosis* and vaccination with BCG. The invention features these polypeptides, functional segments thereof, DNA molecules encoding either the polypeptides or the functional segments, vectors containing the DNA molecules, cells transformed by the vectors, compositions containing one or more of any of the above polypeptides, functional segments, or DNA molecules, and a variety of diagnostic, therapeutic, and prophylactic (vaccine) methodologies utilizing the foregoing.

Specifically, the invention features an isolated DNA molecule containing a DNA sequence encoding a polypeptide with a first amino acid sequence that can be the amino acid sequence of the polypeptide MTBN1, MTBN2, MTBN3, MTBN4, MTBN5, MTBN6, MTBN7 or MTBN8, as depicted in Fig. 1, or a second amino acid sequence identical to the first amino acid sequence with conservative substitutions; the polypeptide has *Mycobacterium tuberculosis* specific antigenic and immunogenic properties. Also included in the invention is an isolated portion of the above DNA molecule. The portion of the DNA molecule encodes a segment of the polypeptide shorter than the full-length polypeptide, and the segment has *Mycobacterium tuberculosis* specific antigenic and immunogenic properties. Other embodiments of the invention are vectors containing the above DNA molecules and transcriptional and translational regulatory sequences operationally linked to the DNA sequence; the regulatory sequences allow for expression of the polypeptide or functional segment encoded by the DNA sequence in a cell. The invention encompasses cells (e.g., eukaryotic and prokaryotic cells) transformed with the above vectors.

The invention encompasses compositions containing any of the above vectors and a pharmaceutically acceptable diluent or filler. Other compositions (to be

used, for example, as DNA vaccines) can contain at least two (e.g., three, four, five, six, seven, eight, nine, ten, twelve, fifteen, or twenty) DNA sequences, each encoding a polypeptide of the *Mycobacterium tuberculosis* complex or a functional segment thereof, with the DNA sequences being operationally linked to transcriptional and translational regulatory sequences which allow for expression of each of the polypeptides in a cell of a vertebrate. In such compositions, at least one (e.g., two, three, four, five, six, seven, or eight) of the DNA sequences is one of the above DNA molecules of the invention. The encoded polypeptides will preferably be those not encoded by the genome of cells of the BCG strain of *M. bovis*.

The invention also features an isolated polypeptide with a first amino acid sequence that can be the sequence of the polypeptide MTBN1, MTBN2, MTBN3, MTBN4, MTBN5, MTBN6, MTBN7 or MTBN8 as depicted in Fig. 1, or a second amino acid sequence identical to the first amino acid sequence with conservative substitutions. The polypeptide has *Mycobacterium tuberculosis* specific antigenic and immunogenic properties. Also included in the invention is an isolated segment of this polypeptide, the segment being shorter than the full-length polypeptide and having *Mycobacterium tuberculosis* specific antigenic and immunogenic properties. Other embodiments are compositions containing the polypeptide, or functional segment, and a pharmaceutically acceptable diluent or filler. Compositions of the invention can also contain at least two (e.g., three, four, five, six, seven, eight, nine, ten, twelve, fifteen, or twenty) polypeptides of the *Mycobacterium tuberculosis* complex, or functional segments thereof, with at least one of the at least two (e.g., two, three, four, five, six, seven, or eight) polypeptides having the sequence of one of the above described polypeptides of the invention. The

polypeptides will preferably be those not encoded by the genome of cells of the BCG strain of *M. bovis*.

The invention also features methods of diagnosis. One embodiment is a method involving: (a) administration of one of the above polypeptide compositions to a subject suspected of having or being susceptible to *Mycobacterium tuberculosis* infection; and (b) detecting an immune response in the subject to the composition, as an indication that the subject has or is susceptible to *Mycobacterium tuberculosis* infection. An example of such a method is a skin test in which the test substance (e.g., compositions containing one or more of MTBN1-MTBN8) is injected intradermally into the subject and in which a skin delayed-type hypersensitivity response is tested for. Another embodiment is a method that involves: (a) providing a population of cells containing CD4 T lymphocytes from a subject; (b) providing a population of cells containing antigen presenting cells (APC) expressing a major histocompatibility complex (MHC) class II molecule expressed by the subject; (c) contacting the CD4 lymphocytes of (a) with the APC of (b) in the presence of one or more of the polypeptides, functional segments, and or polypeptide compositions of the invention; and (d) determining the ability of the CD4 lymphocytes to respond to the polypeptide, as an indication that the subject has or is susceptible to *Mycobacterium tuberculosis* infection. Another diagnostic method of the invention involves: (a) contacting a polypeptide, a functional segment, or a polypeptide/functional segment composition of the invention with a bodily fluid of a subject; (b) detecting the presence of binding of antibody to the polypeptide, functional segment, or polypeptide/functional segment composition, as an indication that the subject has or is susceptible to *Mycobacterium tuberculosis* infection.

Also encompassed by the invention are methods of vaccination. These methods involve administration of any of the above polypeptides, functional segments, or DNA compositions to a subject. The compositions can be administered alone or with one or more of the other compositions.

As used herein, an "isolated DNA molecule" is a DNA which is one or both of: not immediately contiguous with one or both of the coding sequences with which it is immediately contiguous (i.e., one at the 5' end and one at the 3' end) in the naturally-occurring genome of the organism from which the DNA is derived; or which is substantially free of DNA sequence with which it occurs in the organism from which the DNA is derived. The term includes, for example, a recombinant DNA which incorporated into a vector, e.g., into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic fragment produced by PCR or restriction endonuclease treatment) independent of other DNA sequences. Isolated DNA also includes a recombinant DNA which is part of a hybrid DNA encoding additional M. tuberculosis polypeptide sequences.

"DNA molecules" include cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. Where single-stranded, the DNA molecule may be a sense strand or an antisense strand.

An "isolated polypeptide" of the invention is a polypeptide which either has no naturally-occurring counterpart, or has been separated or purified from components which naturally accompany it, e.g., in *M. tuberculosis* bacteria. Typically, the polypeptide is considered "isolated" when it is at least 70%, by dry weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, a preparation of a polypeptide of the

invention is at least 80%, more preferably at least 90%, and most preferably at least 99%, by dry weight, the peptide of the invention. Since a polypeptide that is chemically synthesized is, by its nature, separated from the components that naturally accompany it, the synthetic polypeptide is "isolated."

An isolated polypeptide of the invention can be obtained, for example, by extraction from a natural source (e.g., *M. tuberculosis* bacteria); by expression of a recombinant nucleic acid encoding the polypeptide; or by chemical synthesis. A polypeptide that is produced in a cellular system different from the source from which it naturally originates is "isolated," because it will be separated from components which naturally accompany it. The extent of isolation or purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

The polypeptides may contain a primary amino acid sequence that has been modified from those disclosed herein. Preferably these modifications consist of conservative amino acid substitutions. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine.

The terms "protein" and "polypeptide" are used herein to describe any chain of amino acids, regardless of length or post-translational modification (for example, glycosylation or phosphorylation). Thus, the term "*Mycobacterium tuberculosis* polypeptide" includes full-length, naturally occurring *Mycobacterium tuberculosis* protein, as well a recombinantly or synthetically produced polypeptide that corresponds to a full-length naturally occurring *Mycobacterium tuberculosis* protein or to particular domains or portions

of a naturally occurring protein. The term also encompasses a mature *Mycobacterium tuberculosis* polypeptide which has an added amino-terminal methionine (useful for expression in prokaryotic cells) or any short amino acid sequences useful for protein purification by affinity chromatography, e.g., polyhistidine for purification by metal chelate chromatography.

As used herein, "immunogenic" means capable of activating a primary or memory immune response. Immune responses include responses of CD4+ and CD8+ T lymphocytes and B-lymphocytes. In the case of T lymphocytes, such responses can be proliferative, and/or cytokine (e.g., interleukin(IL)-2, IL-3, IL-4, IL-5, IL-6, IL-12, IL-13, IL-15, tumor necrosis factor-α (TNF-α), or interferon-γ (IFN-γ))-producing, or they can result in generation of cytotoxic T-lymphocytes (CTL). B-lymphocyte responses can be those resulting in antibody production by the responding B lymphocytes.

As used herein, "antigenic" means capable of being recognized by either antibody molecules or antigen-specific T cell receptors (TCR) on activated effector T cells (e.g., cytokine-producing T cells or CTL).

Thus, polypeptides that have "*Mycobacterium tuberculosis* specific antigenic properties" are polypeptides that: (a) can be recognized by and bind to antibodies elicited in response to *Mycobacterium tuberculosis* organisms or wild-type *Mycobacterium tuberculosis* molecules (e.g., polypeptides); or (b) contain subsequences which, subsequent to processing of the polypeptide by appropriate antigen presenting cells (APC) and bound to appropriate major histocompatibility complex (MHC) molecules, are recognized by and bind to TCR on effector T cells elicited in response to *Mycobacterium tuberculosis* organisms or wild-type *Mycobacterium tuberculosis* molecules (e.g., polypeptides).

As used herein, polypeptides that have "*Mycobacterium tuberculosis* specific immunogenic properties" are polypeptides that: (a) can elicit the production of antibodies that recognize and bind to *Mycobacterium tuberculosis* organisms or wild-type *Mycobacterium tuberculosis* molecules (e.g., polypeptides); or (b) contain subsequences which, subsequent to processing of the polypeptide by appropriate antigen presenting cells (APC) and bound to appropriate major histocompatibility complex (MHC) molecules on the surface of the APC, activate T cells with TCR that recognize and bind to peptide fragments derived by processing by APC of *Mycobacterium tuberculosis* organisms or wild-type *Mycobacterium tuberculosis* molecules (e.g., polypeptides) and bound to MHC molecules on the surface of the APC. The immune responses elicited in response to the immunogenic polypeptides are preferably protective. As used herein, "protective" means preventing establishment of an infection or onset of a disease or lessening the severity of a disease existing in a subject. "Preventing" can include delaying onset, as well as partially or completely blocking progress of the disease.

As used herein, a "functional segment of a *Mycobacterium tuberculosis* polypeptide" is a segment of the polypeptide that has *Mycobacterium tuberculosis* specific antigenic and immunogenic properties.

Where a polypeptide, functional segment of a polypeptide, or a mixture of polypeptides and/or functional segments have been administered (e.g., by intradermal injection) to a subject for the purpose of testing for a *M. tuberculosis* infection or susceptibility to such an infection, "detecting an immune response" means examining the subject for signs of a immunological reaction to the administered material, e.g., reddening or swelling of the skin at the site of an intradermal

injection. Where the subject has antibodies to the administered material, the response will generally be rapid, e.g., 1 minute to 24 hours. On the other hand, a memory or activated T cell reaction of pre-immunized T lymphocytes in the subject is generally slower, appearing only after 24 hours and being maximal at 24-96 hours.

As used herein, a "subject" can be a human subject or a non-human mammal such as a non-human primate, a horse, a bovine animal, a pig, a sheep, a goat, a dog, a cat, a rabbit, a guinea pig, a hamster, a rat, or a mouse.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention. Unless otherwise indicated, these materials and methods are illustrative only and are not intended to be limiting. All publications, patent applications, patents and other references mentioned herein are illustrative only and not intended to be limiting.

Other features and advantages of the invention, e.g., methods of diagnosing *M. tuberculosis* infection, will be apparent from the following description, from the drawings and from the claims.

Brief Description of the Drawings

Figure 1 is a depiction of the amino acid sequences of *M. tuberculosis* polypeptides MTBN1-MTBN8.

Figure 2 is a depiction of the nucleotide sequences of the coding regions (mtbn1-mtbn8) encoding MTBN1-MTBN8.

Figure 3 is a bar graph showing the delayed-type hypersensitivity responses induced by intradermal injection of 3 different test reagents in female guinea pigs that had been either infected with *M. tuberculosis* cells or sensitized with BCG or *M. avium* cells.

Detailed Description

The genome of *M. tuberculosis* [Cole et al. (1998) Nature 393:537-544] contains open reading frames (ORFs) that have been deleted from the avirulent BCG strain. The polypeptides encoded by these ORFs are designated herein "*M. tuberculosis* BCG Negative" polypeptides ("MTBN") and the ORFs are designated "mtbn." The invention is based on the discovery that a MTBN polypeptide (MTBN4) elicited a skin response in animals infected with *M. tuberculosis*, but not in animals sensitized to either BCG or *M. avium*, a non-*M. tuberculosis*-complex strain of mycobacteria (see Example 1 below). These findings indicate that MTBN (e.g., MTBN1-MTBN8) can be used in diagnostic tests that discriminate infection of a subject by *M. tuberculosis* from exposure to both mycobacteria other than the *M. tuberculosis*-complex and BCG. The *M. tuberculosis*-complex includes *M. tuberculosis*, *M. bovis*, *M. microti*, and *M. africanum*. Thus they can be used to discriminate subjects exposed to *M. tuberculosis*, and thus potentially having or being in danger of having tuberculosis, from subjects that have been vaccinated with BCG, the most widely used tuberculosis vaccine. Diagnostic assays that are capable of such discrimination represent a major advance that will greatly reduce wasted effort and consequent costs resulting from further diagnostic tests and/or therapeutic procedures in subjects that have given positive results in less discriminatory diagnostic tests. Furthermore, the results in Example 1 show that MTBN4, as expressed by whole viable *M. tuberculosis* organisms, is capable of inducing a strong immune response in subjects

infected with the organisms and thus has the potential to be a vaccine.

The MTBN polypeptides of the invention include, for example, polypeptides encoded within the RD1, RD2, and RD3 regions of the *M. tuberculosis* genome [Mahairas et al. (1996) J. Bacteriol. 178:1274-1282]. Of particular interest are polypeptides encoded by ORFs within the RD1 region of the *M. tuberculosis* genome. However, the invention is not restricted to the RD1, RD2, and RD3 region encoded polypeptides and includes any polypeptides encoded by ORFs contained in the genome of one or more members of the *M. tuberculosis* genome and not contained in the genome of BCG. The amino acid sequences of MTBN1-MTBN8 are shown in Fig. 1 and the nucleotide sequences of mtbn1-mtbn8 are shown in Fig. 2.

The invention encompasses: (a) isolated DNA molecules containing mtbn sequences (e.g., mtbn1-mtbn8) encoding MTBN polypeptides (e.g., MTBN1-MTBN8) and isolated portions of such DNA molecules that encode polypeptide segments having antigenic and immunogenic properties (i.e., functional segments); (b) the MTBN polypeptides themselves (e.g., MTBN1-MTBN8) and functional segments of them; (c) antibodies (including antigen binding fragments, e.g., $F(ab')_2$, Fab, Fv, and single chain Fv fragments of such antibodies) that bind to the MTBN polypeptides (e.g., MTBN1-MTBN8) and functional segments; (d) nucleic acid molecules (e.g., vectors) containing and capable of expressing one or more of the mtbn (e.g., mtbn1-mtbn8) sequences and portions of DNA molecules; (e) cells (e.g., bacterial, yeast, insect, or mammalian cells) transformed by such vectors; (f) compositions containing vectors encoding one or more *M. tuberculosis* polypeptides (or functional segments) including both the MTBN (e.g., MTBN1-MTBN8) polypeptides (or functional segments thereof) and previously described *M. tuberculosis* polypeptides such as ESAT-6, 14 kDa

antigen, MPT63, 19 kDa antigen, MPT64, MPT51, MTC28, 38 kDa antigen, 45/47 kDa antigen, MPB70, Ag85 complex, MPT53, and KatG (see also U.S. application no. 08/796,792); (g) compositions containing one or more *M. tuberculosis* polypeptides (or functional segments), including both the polypeptides of the invention and previously described *M. tuberculosis* polypeptides such as those described above; (h) compositions containing one or more of the antibodies described in (c); (i) methods of diagnosis involving either (1) administration (e.g., intradermal injection) of any of the above polypeptide compositions to a subject suspected of having or being susceptible to *M. tuberculosis* infection, (2) *in vitro* testing of lymphocytes (B-lymphocytes, CD4 T lymphocytes, and CD8 T lymphocytes) from such a subject for responsiveness (e.g., by measuring cell proliferation, antibody production, cytokine production, or CTL activity) to any of the above polypeptide compositions, (3) testing of a bodily fluid (e.g., blood, saliva, plasma, serum, urine, or semen or a lavage such as a bronchoalveolar lavage, a vaginal lavage, or lower gastrointestinal lavage) for antibodies to the MTBN polypeptides (e.g., MTBN1-MTBN8) or functional segments thereof, or the above-described polypeptide compositions; (4) testing of a bodily fluid (e.g., as above) for the presence of *M. tuberculosis*, MTBN (e.g., MTBN1-MTBN8) polypeptides or functional segments thereof, or the above-described polypeptide compositions in assays using the antibodies described in (c); and (5) testing of a tissue (e.g., lung or bronchial tissue) or a body fluid (e.g., as above) for the presence of nucleic acid molecules (e.g., DNA or RNA) encoding MTBN polypeptides (e.g., MTBN1-MTBN8) (or portions of such a nucleic acid molecules) using nucleic acid probes or primers having nucleotide sequences of the nucleic molecules, portions of the nucleic molecules, or the complements of such

molecules; and (j) methods of vaccination involving administration to a subject of the compositions of either (f), (g), (h) or a combination of any two or even all 3 compositions.

With respect to diagnosis, purified MTBN proteins, functional segments of such proteins, or mixtures of proteins and/or the functional fragments have the above-described advantages of discriminating infection by *M. tuberculosis* from either infection by other bacteria, and in particular, non-pathogenic mycobacteria, or from exposure (by, for example, vaccination) to BCG. Furthermore, compositions containing the proteins, functional segments of the proteins, or mixtures of the proteins and/or the functional segments allows for improved quality control since "batch-to-batch" variability is greatly reduced in comparison to complex mixtures such as purified protein derivative (PPD) of tuberculin.

The use of the above-described polypeptide and nucleic acid reagents for vaccination also provides for highly specific and effective immunization. Since the virulent *M. tuberculosis* polypeptides encoded by genes absent from avirulent BCG are likely to be mediators of virulence, immunity directed to them can be especially potent in terms of protective capacity. Where vaccination is performed with nucleic acids both *in vivo* and *ex vivo* methods can be used. *In vivo* methods involve administration of the nucleic acids themselves to the subject and *ex vivo* methods involve obtaining cells (e.g., bone marrow cells or fibroblasts) from the subject, transducing the cells with the nucleic acids, preferably selecting or enriching for successfully transduced cells, and administering the transduced cells to the subject. Alternatively, the cells that are transduced and administered to the subject can be derived from another subject. Methods of vaccination and

diagnosis are described in greater detail in U.S. application no. 08/796,792 which is incorporated herein by reference in its entirety.

The following example is meant to illustrate, not limit the invention.

Example 1. MPBN4 Elicits a Specific Skin Reaction in Guinea Pigs Infected with *M. tuberculosis*

Four groups of outbred female guinea pigs (18 per group) were used to test the usefulness of the MTBN4 polypeptide as a *M. tuberculosis*-specific diagnostic reagent. The four groups were treated as follows.

Group 1 animals were infected by aerosol with approximately 100 *M. tuberculosis strain H37Rv* cells.
Group 2 animals were sensitized intradermally with 10^6 live *M. bovis* BCG Japanese cells.
Group 3 animals were sensitized intradermally with 10^6 live *M. avium* cells.
Group 4 animals were mock-sensitized by intradermal injection with saline.

Seven weeks after infection or sensitization, the animals were injected intradermally with 1 µg of PPD (6 animals from each group), 2 µg of purified recombinant MPT64 (6 animals from each group), or 2 µg of MTBN4 (6 animals from each group). The diameter of the resulting erythema was measured 24 hours later. Data are expressed as mean diameter of erythema (in mm) and standard deviations are indicated (Fig. 3).

No erythema was detected in the group 4 animals with any test substance and thus no data are shown for this group. On the other hand, group 1 animals (solid bars) showed a significant response with all three test substances. Group 2 animals (open bars) showed a significant response to PPD and MPT64 but not MTBN4.

Group 3 animals showed a significant response to PPD only (hatched bars).

Thus, PPD which contains antigenic/immunogenic molecules common to the *M. tuberculosis*-complex as well as other mycobacterial strains, gave the least discriminatory results in that it induced responses in animals infected with or sensitized to mycobacteria of the *M. tuberculosis*-complex (*M. tuberculosis* and BCG) as well as another non-pathogenic mycobacterium (*M. avium*). While MPT64, which is encoded and expressed by both *M. tuberculosis* and BCG, did not elicit a response in animals infected with *M. avium*, it did elicit responses in both the *M. tuberculosis* infected and the BCG sensitized animals. Finally, MTBN4 elicited a response in only the *M. tuberculosis* animals. Thus it induced the most specific response and, most importantly, allowed for discrimination between animals infected with *M. tuberculosis* and those sensitized to BCG.

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1. An isolated DNA molecule comprising a DNA sequence encoding a polypeptide with a first amino acid sequence selected from the group consisting of the amino acid sequences of the polypeptides MTBN1, MTBN2, MTBN3, MTBN4, MTBN5, MTBN6, MTBN7, and MTBN8, as depicted in Fig. 1,

or a second amino acid sequence identical to said first amino acid sequence with conservative substitutions,

wherein said polypeptide has *Mycobacterium tuberculosis* specific antigenic and immunogenic properties.

2. An isolated portion of the DNA molecule of claim 1, said portion encoding a segment of said polypeptide shorter than the full-length polypeptide, said segment having *Mycobacterium tuberculosis* specific antigenic and immunogenic properties.

3. A vector comprising:

(a) the DNA molecule of claim 1; and

(b) transcriptional and translational regulatory sequences operationally linked to said DNA sequence, said regulatory sequences allowing for expression of the polypeptide encoded by said DNA sequence in a cell.

4. A vector comprising:

(a) the DNA molecule of claim 2; and

(b) transcriptional and translational regulatory sequences operationally linked to said DNA sequence, said regulatory sequences allowing for expression of the polypeptide encoded by said DNA sequence in a cell.

5. A cell transformed with the vector of claim 3.

6. A cell transformed with the vector of claim 4.

7. A composition comprising the vector of claim 3 and a pharmaceutically acceptable diluent or filler.

8. A composition comprising the vector of claim 4 and a pharmaceutically acceptable diluent or filler.

9. A composition comprising at least two DNA sequences, each encoding a polypeptide of the *Mycobacterium tuberculosis* complex that is not a polypeptide encoded by the genome of cells of the Bacille Calmette Guerin (BCG) strain of *Mycobacteria bovis*, said DNA sequences being operationally linked to transcriptional and translational regulatory sequences which allow for expression of each said polypeptide in a cell of a vertebrate,

wherein at least one of said DNA sequences is a DNA molecule of claim 1.

10. A composition comprising at least two DNA sequences, each encoding a functional fragment of a polypeptide of the *Mycobacterium tuberculosis* complex, said DNA sequences being operationally linked to transcriptional and translational regulatory sequences which allow for expression of each said polypeptide in a cell of a vertebrate,

wherein at least one of said DNA sequences is a DNA molecule of claim 2.

11. An isolated polypeptide with a first amino acid sequence selected from the group consisting of the sequences of the polypeptides MTBN1, MTBN2, MTBN3, MTBN4, MTBN5, MTBN6, MTBN7, and MTBN8, as depicted in Fig. 1,

or a second amino acid sequence identical to said first amino acid sequence with conservative substitutions,

wherein said polypeptide has *Mycobacterium tuberculosis* specific antigenic and immunogenic properties.

12. An isolated segment of the polypeptide of claim 11, said segment being shorter than the full-length polypeptide and having *Mycobacterium tuberculosis* specific antigenic and immunogenic properties.

13. A composition comprising the polypeptide of claim 11 and a pharmaceutically acceptable diluent or filler.

14. A composition comprising a functional fragment of the polypeptide of claim 12 and a pharmaceutically acceptable diluent or filler.

15. A composition comprising at least two polypeptides of the *Mycobacterium tuberculosis* complex, each polypeptide not being encoded by the genome of the cells of the BCG strain of *Mycobacterium bovis*, wherein at least one of said polypeptides is a polypeptide of claim 1.

16. A composition comprising functional fragments of at least two polypeptides of the *Mycobacterium tuberculosis* complex, each polypeptide not being encoded by the genome of cells of the Bacille Calmette Guerin (BCG) strain of *Mycobacteria bovis*, wherein at least one of said polypeptides is a segment of claim 2.

17. A method of diagnosis comprising:

(a) administration of the composition of claim 15 to a subject suspected of having or being susceptible to *Mycobacterium tuberculosis* infection; and

(b) detecting an immune response in said subject to said composition as an indication that said subject has or is susceptible to *Mycobacterium tuberculosis* infection.

18. A method of diagnosis comprising:

(a) administration of the composition of claim 16 to a subject suspected of having or being susceptible to *Mycobacterium tuberculosis* infection; and

(b) detecting an immune response in said subject to said composition as an indication that said subject has or is susceptible to *Mycobacterium tuberculosis* infection.

19. A method of diagnosis comprising:

(a) providing a population of cells comprising CD4 T lymphocytes from a subject;

(b) providing a population of cells comprising antigen presenting cells (APC) expressing a major histocompatibility complex (MHC) class II molecule expressed by said subject;

(c) contacting the CD4 lymphocytes of (a) with the APC of (b) in the presence of the polypeptide of claim 12; and

(d) determining the ability of said CD4 lymphocytes to respond to said polypeptide, as an indication that said subject has or is susceptible to *Mycobacterium tuberculosis* infection.

20. A method of diagnosis comprising:

(a) providing a population of cells comprising CD4 T lymphocytes from a subject;

(b) providing a population of cells comprising antigen presenting cells (APC) expressing at least one major histocompatibility complex (MHC) class II molecule expressed by said subject;

(c) contacting the CD4 lymphocytes of (a) with the APC of (b) in the presence of the segment of claim 12; and

(d) determining the ability of said CD4 lymphocytes to respond to said polypeptide, as an

indication that said subject has or is susceptible to *Mycobacterium tuberculosis* infection.

21. A method of diagnosis comprising:

(a) providing a population of cells comprising CD4 T lymphocytes from a subject;

(b) providing a population of cells comprising antigen presenting cells (APC) expressing at least one major histocompatibility complex (MHC) class II molecule expressed by said subject;

(c) contacting the CD4 lymphocytes of (a) with the APC of (b) in the presence of the composition of claim 15; and

(d) determining the ability of said CD4 lymphocytes to respond to said polypeptide, as an indication that said subject has or is susceptible to *Mycobacterium tuberculosis* infection.

22. A method of diagnosis comprising:

(a) providing a population of cells comprising CD4 T lymphocytes from a subject;

(b) providing a population of cells comprising antigen presenting cells (APC) expressing at least one major histocompatibility complex (MHC) class II molecule expressed by said subject;

(c) contacting the CD4 lymphocytes of (a) with the APC of (b) in the presence of the composition of claim 16; and

(d) determining the ability of said CD4 lymphocytes to respond to said polypeptide, as an indication that said subject has or is susceptible to *Mycobacterium tuberculosis* infection.

23. A method of diagnosis comprising:

(a) contacting the polypeptide of claim 11 with a bodily fluid of a subject;

(b) detecting the presence of binding of antibody to said polypeptide, as an indication that said subject has or is susceptible to *Mycobacterium tuberculosis* infection.

24. A method of diagnosis comprising:

(a) contacting the segment of claim 12 with a bodily fluid of a subject;

(b) detecting the presence of binding of antibody to said polypeptide, as an indication that said subject has or is susceptible to *Mycobacterium tuberculosis* infection.

25. A method of diagnosis comprising:

(a) contacting the composition of claim 15 with a bodily fluid of a subject;

(b) detecting the presence of binding of antibody to said composition, as an indication that said subject has or is susceptible to *Mycobacterium tuberculosis* infection.

26. A method of diagnosis comprising:

(a) contacting the composition of claim 16 with a bodily fluid of a subject;

(b) detecting the presence of binding of antibody to said composition, as an indication that said subject has or is susceptible to *Mycobacterium tuberculosis* infection.

27. A method of vaccination comprising administration of the composition of claim 7 to a subject.

28. A method of vaccination comprising administration of the composition of claim 8 to a subject.

29. A method of vaccination comprising administration of the composition of claim 9 to a subject.

30. A method of vaccination comprising administration of the composition of claim 10 to a subject.

31. A method of vaccination comprising administration of the composition of claim 13 to a subject.

32. A method of vaccination comprising administration of the composition of claim 14 to a subject.

33. A method of vaccination comprising administration of the composition of claim 15 to a subject.

34. A method of vaccination comprising administration of the composition of claim 16 to a subject.

FIG. 1

MTBN1
MTAEPEVRTLREVVLDQLGTAESRAYKMWLPPLTNPVPLNELIARDRRQPLRFALGIMDE
PRRHLQDVWGVDVSGAGGNIGIGGAPQTGKSTLLQTMVMSAAATHSPRNVQFYCIDLGGG
GLIYLENLPHVGGVANRSEPDKVNRVVAEMQAVMRQRETTFKEHRVGSIGMYRQLRDDPS
QPVASDPYGDVFLIIDGWPGFVGEFPDLEGQVQDLAAQGLAFGVHVIISTPRWTELKSRV
RDYLGTKIEFRLGDVNETQIDRITREIPANRPGRAVSMEKHHLMIGVPRFDGVHSADNLV
EAITAGVTQIASQHTEQAPPVRVLPERIHLHELDPNPPGPESDYRTRWEIPIGLRETDLT
PAHCHMHTNPHLLIFGAAKSGKTTIAHAIARAICARNSPQQVRFMLADYRSGLLDAVPDT
HLLGAGAINRNSASLDEAVQALAVNLKKRLPPTDLTTAQLRSRSWWSGFDVVLLVDDWHM
IVGAAGGMPPMAPLAPLLPAAADIGLHIIVTCQMSQAYKATMDKFVGAAFGSGAPTMFLS
GEKQEFPSSEFKVKRRPPGQAFLVSPDGKEVIQAPYIEPPEEVFAAPPSAG*

MTBN2
MEKMSHDPIAADIGTQVSDNALHGVTAGSTALTSVTGLVPAGADEVSAQAATAFTSEGIQ
LLASNASAQDQLHRAGEAVQDVARTYSQIDDGAAGVFAE*

MTBN3
MLWHAMPPELNTARLMAGAGPAPMLAAAAGWQTLSAALDAQAVELTARLNSLGEAWTGGG
SDKALAAATPMVVWLQTASTQAKTRAMQATAQAAAYTQAMATTPSLPEIAANHITQAVLT
ATNFFGINTIPIALTEMDYFIRMWNQAALAMEVYQAETAVNTLFEKLEPMASILDPGASQ
STTNPIFGMPSPGSSTPVGQLPPAATQTLGQLGEMSGPMQQLTQPLQQVTSLFSQVGGTG
GGNPADEEAAQMGLLGTSPLSNHPLAGGSGPSAGAGLLRAESLPGAGGSLTRTPLMSQLI
EKPVAPSVMPAAAAGSSATGGAAPVGAGAMGQGAQSGGSTRPGLVAPAPLAQEREEDDED
DWDEEDDW*

MTBN4
MAEMKTDAATLAQEAGNFERISGDLKTQIDQVESTAGSLQGQWRGAAGTAAQAAVVRFQE
AANKQKQELDEISTNIRQAGVQYSRADEEQQQALSSQMGF*

MTBN5
MAADYDKLFRPHEGMEAPDDMAAQPFFDPSASFPPAPASANLPKPNGQTPPPTSDDLSER
FVSAPPPPPPPPPPPPPTPMPIAAGEPPSPEPAASKPPTPPMPIAGPEPAPPKPPTPPMP
IAGPEPAPPKPPTPPMPIAGPAPTPTESQLAPPRPPTPQTPTGAPQQPESPAPHVPSHGP
HQPRRTAPAPPWAKMPIGEPPPAPSRPSASPAEPPTRPAPQHSRRARRGHRYRTDTERNV
GKVATGPSIQARLRAEEASGAQLAPGTEPSPAPLGQPRSYLAPPTRPAPTEPPPSPSPQR
NSGRRAERRVHPDLAAQHAAAQPDSITAATTGGRRRKRAAPDLDATQKSLRPAAKGPKVK
KVKPQKPKATKPPKVVSQRGWRHWVHALTRINLGLSPDEKYELDLHARVRRNPRGSYQIA
VVGLKGGAGKTTLTAALGSTLAQVRADRILALDADPGAGNLADRVGRQSGATIADVLAEK
ELSHYNDIRAHTSVNAVNLEVLPAPEYSSAQRALSDADWHFIADPASRFYNLVLADCGAG
FFDPLTRGVLSTVSGVVVVASVSIDGAQQASVALDWLRNNGYQDLASRACVVINHIMPGE
PNVAVKDLVRHFEQQVQPGRVVVMPWDRHIAAGTEISLDLLDPIYKRKVLELAAALSDDF
ERAGRR*

FIG. 1 (continued)

MTBN6
LSAPAVAAGPTAAGATAARPATTRVTILTGRRMTDLVLPAAVPMETYIDDTVAVLSEVLE
DTPADVLGGFDFTAQGVWAFARPGSPPLKLDQSLDDAGVVDGSLLTLVSVSRTERYRPLV
EDVIDAIAVLDESPEFDRTALNRFVGAAIPLLTAPVIGMAMRAWWETGRSLWWPLAIGIL
GIAVLVGSFVANRFYQSGHLAECLLVTTYLLIATAAALAVPLPRGVNSLGAPQVAGAATA
VLFLTLMTRGGPRKRHELASFAVITAIAVIAAAAAFGYGYQDWVPAGGIAFGLFIVTNAA
KLTVAVARIALPPIPVPGETVDNEELLDPVATPEATSEETPTWQAIIASVPASAVRLTER
SKLAKQLLIGYVTSGTLILAAGAIAVVVRGHFFVHSLVVAGLITTVCGFRSRLYAERWCA
WALLAATVAIPTGLTAKLIIWYPHYAWLLLSVYLTVALVALVVVGSMAHVRRVSPVVKRT
LELIDGAMIAAIIPMLLWITGVYDTVRNIRF*

MTBN7
MAEPLAVDPTGLSAAAAKLAGLVFPQPPAPIAVSGTDSVVAAINETMPSIESLVSDGLPG
VKAALTRTASNMNAAADVYAKTDQSLGTSLSQYAFGSSGEGLAGVASVGGQPSQATQLLS
TPVSQVTTQLGETAAELAPRVVATVPQLVQLAPHAVQMSQNASPIAQTISQTAQQAAQSA
QGGSGPMPAQLASAEKPATEQAEPVHEVTNDDQGDQGDVQPAEVVAAARDEGAGASPGQQ
PGGGVPAQAMDTGAGARPAASPLAAPVDPSTPAPSTTTTL*

MTBN8
MSITRPTGSYARQMLDPGGWVEADEDTFYDRAQEYSQVLQRVTDVLDTCRQQKGHVFEGG
LWSGGAANAANGALGANINQLMTLQDYLATVITWHRHIAGLIEQAKSDIGNNVDGAQREI
DILENDPSLDADERHTAINSLVTATHGANVSLVAETAERVLESKNWKPPKNALEDLLQQK
SPPPPDVPTLVVPSPGTPGTPGTPITPGTPITPGTPITPIPGAPVTPITPTPGTPVTPVT
PGKPVTPVTPVKPGTPGEPTPITPVTPPVAPATPATPATPVTPAPAPHPQPAPAPAPSPG
PQPVTPATPGPSGPATPGTPGGEPAPHVKPAALAEQPGVPGQHAGGGTQSGPAHADESAA
SVTPAAASGVPGARAAAAAPSGTAVGAGARSSVGTAAASGAGSHAATGRAPVATSDKAAA
PSTRAASARTAPPARPPSTDHIDKPDRSESADDGTPVSMIPVSAARAARDAATAAASARQ
RGRGDALRLARRIAAALNASDNNAGDYGFFWITAVTTDGSIVVANSYGLAYIPDGMELPN
KVYLASADHAIPVDEIARCATYPVLAVQAWAAFHDMTLRAVIGTAEQLASSDPGVAKIVL
EPDDIPESGKMTGRSRLEVVDPSAAAQLADTTDQRLLDLLPPAPVDVNPPGDERHMLWFE
LMKPMTSTATGREAAHLRAFRAYAAHSQEIALHQAHTATDAAVQRVAVADWLYWQYVTGL
LDRALAAAC*

FIG. 2

mtbn1

```
1     atgactgctg aaccggaagt acggacgctg cgcgaggttg tgctggacca
51    gctcggcact gctgaatcgc gtgcgtacaa gatgtggctg ccgccgttga
101   ccaatccggt cccgctcaac gagctcatcg cccgtgatcg gcgacaaccc
151   ctgcgatttg ccctggggat catggatgaa ccgcgccgcc atctacagga
201   tgtgtggggc gtagacgttt ccggggccgg cggcaacatc ggtattgggg
251   gcgcacctca aaccgggaag tcgacgctac tgcagacgat ggtgatgtcg
301   gccgccgcca cacactcacc gcgcaacgtt cagttctatt gcatcgacct
351   aggtggcggc gggctgatct atctcgaaaa ccttccacac gtcggtgggg
401   tagccaatcg gtccgagccc gacaaggtca accgggtggt cgcagagatg
451   caagccgtca tgcggcaacg ggaaaccacc ttcaaggaac accgagtggg
501   ctcgatcggg atgtaccggc agctgcgtga cgatccaagt caacccgttg
551   cgtccgatcc atacggcgac gtctttctga tcatcgacgg atggcccggt
601   tttgtcggcg agttccccga ccttgagggg caggttcaag atctggccgc
651   ccaggggctg gcgttcggcg tccacgtcat catctccacg ccacgctgga
701   cagagctgaa gtcgcgtgtt cgcgactacc tcggcaccaa gatcgagttc
751   cggcttggtg acgtcaatga aacccagatc gaccggatta cccgcgagat
801   cccggcgaat cgtccgggtc gggcagtgtc gatggaaaag caccatctga
851   tgatcggcgt gcccaggttc gacggcgtgc acagcgccga taacctggtg
901   gaggcgatca ccgcgggggt gacgcagatc gcttcccagc acaccgaaca
951   ggcacctccg gtgcgggtcc tgccggagcg tatccacctg cacgaactcg
1001  acccgaaccc gccgggacca gagtccgact accgcactcg ctgggagatt
1051  ccgatcggct tgcgcgagac ggacctgacg ccggctcact gccacatgca
1101  cacgaacccg cacctactga tcttcggtgc ggccaaatcg ggcaagacga
1151  ccattgccca cgcgatcgcg cgcgccattt gtgcccgaaa cagtccccag
1201  caggtgcggt tcatgctcgc ggactaccgc tcgggcctgc tggacgcggt
1251  gccggacacc catctgctgg gcgccggcgc gatcaaccgc aacagcgcgt
1301  cgctagacga ggccgttcaa gcactggcgg tcaacctgaa gaagcggttg
1351  ccgccgaccg acctgacgac ggcgcagcta cgctcgcgtt cgtggtggag
1401  cggatttgac gtcgtgcttc tggtcgacga ttggcacatg atcgtgggtg
1451  ccgccggggg gatgccgccg atggcaccgc tggccccgtt attgccggcg
1501  gcggcagata tcgggttgca catcattgtc acctgtcaga tgagccaggc
1551  ttacaaggca accatggaca agttcgtcgg cgccgcattc gggtcgggcg
1601  ctccgacaat gttcctttcg ggcgagaagc aggaattccc atccagtgag
1651  ttcaaggtca agcggcgccc ccctggccag gcatttctcg tctcgccaga
1701  cggcaaagag gtcatccagg ccccctacat cgagcctcca gaagaagtgt
1751  tcgcagcacc cccaagcgcc ggttaa
```

mtbn2

```
1     atggaaaaaa tgtcacatga tccgatcgct gccgacattg gcacgcaagt
51    gagcgacaac gctctgcacg gcgtgacggc cggctcgacg gcgctgacgt
101   cggtgaccgg gctggttccc gcgggggccg atgaggtctc cgcccaagcg
151   gcgacggcgt tcacatcgga gggcatccaa ttgctggctt ccaatgcatc
201   ggcccaagac cagctccacc gtgcgggcga agcggtccag gacgtcgccc
251   gcacctattc gcaaatcgac gacggcgccg ccggcgtctt cgcctaatag
```

mtbn3

```
1     atgctgtggc acgcaatgcc accggagcta aataccgcac ggctgatggc
51    cggcgcgggt ccggctccaa tgcttgcggc ggccgcggga tggcagacgc
101   tttcggcggc tctggacgct caggccgtcg agttgaccgc gcgcctgaac
```

FIG. 2 (continued)

```
151  tctctgggag aagcctggac tggaggtggc agcgacaagg cgcttgcggc
201  tgcaacgccg atggtggtct ggctacaaac cgcgtcaaca caggccaaga
251  cccgtgcgat gcaggcgacg gcgcaagccg cggcatacac ccaggccatg
301  gccacgacgc cgtcgctgcc ggagatcgcc gccaaccaca tcacccaggc
351  cgtccttacg gccaccaact tcttcggtat caacacgatc ccgatcgcgt
401  tgaccgagat ggattatttc atccgtatgt ggaaccaggc agccctggca
451  atggaggtct accaggccga gaccgcggtt aacacgcttt tcgagaagct
501  cgagccgatg gcgtcgatcc ttgatcccgg cgcgagccag agcacgacga
551  acccgatctt cggaatgccc tcccctggca gctcaacacc ggttggccag
601  ttgccgccgg cggctaccca gaccctcggc caactgggtg agatgagcgg
651  cccgatgcag cagctgaccc agccgctgca gcaggtgacg tcgttgttca
701  gccaggtggg cggcaccggc ggcggcaacc cagccgacga ggaagccgcg
751  cagatgggcc tgctcggcac cagtccgctg tcgaaccatc cgctggctgg
801  tggatcaggc cccagcgcgg gcgcgggcct gctgcgcgcg gagtcgctac
851  ctggcgcagg tgggtcgttg acccgcacgc cgctgatgtc tcagctgatc
901  gaaaagccgg ttgccccctc ggtgatgccg gcggctgctg ccggatcgtc
951  ggcgacgggt ggcgccgctc cggtgggtgc gggagcgatg ggccagggtg
1001 cgcaatccgg cggctccacc aggccgggtc tggtcgcgcc ggcaccgctc
1051 gcgcaggagc gtgaagaaga cgacgaggac gactgggacg aagaggacga
1101 ctggtga
```

mtbn4

```
1    atggcagaga tgaagaccga tgccgctacc ctcgcgcagg aggcaggtaa
51   tttcgagcgg atctccggcg acctgaaaac ccagatcgac caggtggagt
101  cgacggcagg ttcgttgcag ggccagtggc gcggcgcggc ggggacggcc
151  gcccaggccg cggtggtgcg cttccaagaa gcagccaata agcagaagca
201  ggaactcgac gagatctcga cgaatattcg tcaggccggc gtccaatact
251  cgagggccga cgaggagcag cagcaggcgc tgtcctcgca aatgggcttc
301  tga
```

mtbn5

```
1    atggcggccg actacgacaa gctcttccgg ccgcacgaag gtatggaagc
51   tccggacgat atggcagcgc agccgttctt cgaccccagt gcttcgtttc
101  cgccggcgcc cgcatcggca aacctaccga agcccaacgg ccagactccg
151  cccccgacgt ccgacgacct gtcggagcgg ttcgtgtcgg ccccgccgcc
201  gccacccccа cccccacctc cgcctccgcc aactccgatg ccgatcgccg
251  caggagagcc gccctcgccg gaaccggccg catctaaacc acccacaccc
301  cccatgccca tcgccggacc cgaaccggcc ccacccaaac cacccacacc
351  ccccatgccc atcgccggac ccgaaccggc cccacccaaa ccacccacac
401  ctccgatgcc catcgccgga cctgcaccca ccccaaccga atcccagttg
451  gcgcccccca gaccaccgac accacaaacg ccaaccggag cgccgcagca
501  accggaatca ccggcgcccc acgtaccctc gcacgggcca catcaacccc
551  ggcgcaccgc accagcaccg ccctgggcaa agatgccaat cggcgaaccc
601  ccgcccgctc cgtccagacc gtctgcgtcc ccggccgaac caccgacccg
651  gcctgccccc caacactccc gacgtgcgcg ccggggtcac cgctatcgca
701  cagacaccga acgaaacgtc gggaaggtag caactggtcc atccatccag
751  gcgcggctgc gggcagagga agcatccggc gcgcagctcg cccccggaac
801  ggagccctcg ccagcgccgt tgggccaacc gagatcgtat ctggctccgc
851  ccacccgccc cgcgccgaca gaacctcccc ccagcccctc gccgcagcgc
901  aactccggtc ggcgtgccga gcgacgcgtc caccccgatt tagccgccca
```

FIG. 2 (continued)

```
951  acatgccgcg gcgcaacctg attcaattac ggccgcaacc actggcggtc
1001 gtcgccgcaa gcgtgcagcg ccggatctcg acgcgacaca gaaatcctta
1051 aggccggcgg ccaaggggcc gaaggtgaag aaggtgaagc cccagaaacc
1101 gaaggccacg aagccgccca aagtggtgtc gcagcgcggc tggcgacatt
1151 gggtgcatgc gttgacgcga atcaacctgg gcctgtcacc cgacgagaag
1201 tacgagctgg acctgcacgc tcgagtccgc cgcaatcccc gcgggtcgta
1251 tcagatcgcc gtcgtcggtc tcaaaggtgg ggctggcaaa accacgctga
1301 cagcagcgtt ggggtcgacg ttggctcagg tgcgggccga ccggatcctg
1351 gctctagacg cggatccagg cgccggaaac ctcgccgatc gggtagggcg
1401 acaatcgggc gcgaccatcg ctgatgtgct tgcagaaaaa gagctgtcgc
1451 actacaacga catccgcgca cacactagcg tcaatgcggt caatctggaa
1501 gtgctgccgg caccggaata cagctcggcg cagcgcgcgc tcagcgacgc
1551 cgactggcat ttcatcgccg atcctgcgtc gaggttttac aacctcgtct
1601 tggctgattg tggggccggc ttcttcgacc cgctgacccg cggcgtgctg
1651 tccacggtgt ccggtgtcgt ggtcgtggca agtgtctcaa tcgacggcgc
1701 acaacaggcg tcggtcgcgt tggactggtt gcgcaacaac ggttaccaag
1751 atttggcgag ccgcgcatgc gtggtcatca atcacatcat gccgggagaa
1801 cccaatgtcg cagttaaaga cctggtgcgg catttcgaac agcaagttca
1851 acccggccgg gtcgtggtca tgccgtggga caggcacatt gcggccggaa
1901 ccgagatttc actcgacttg ctcgacccta tctacaagcg caaggtcctc
1951 gaattggccg cagcgctatc cgacgatttc gagagggctg gacgtcgttg
2001  a
```

mtbn6

```
1    ttgagcgcac ctgctgttgc tgctggtcct accgccgcgg gggcaaccgc
51   tgcgcggcct gccaccaccc gggtgacgat cctgaccggc agacggatga
101  ccgatttggt actgccagcg gcggtgccga tggaaactta tattgacgac
151  accgtcgcgg tgctttccga ggtgttggaa gacacgccgg ctgatgtact
201  cggcggcttc gactttaccg cgcaaggcgt gtgggcgttc gctcgtcccg
251  gatcgccgcc gctgaagctc gaccagtcac tcgatgacgc cggggtggtc
301  gacgggtcac tgctgactct ggtgtcagtc agtcgcaccg agcgctaccg
351  accgttggtc gaggatgtca tcgacgcgat cgccgtgctt gacgagtcac
401  ctgagttcga ccgcacggca ttgaatcgct ttgtgggggc ggcgatcccg
451  cttttgaccg cgcccgtcat cgggatggcg atgcgggcgt ggtgggaaac
501  tgggcgtagc ttgtggtggc cgttggcgat tggcatcctg gggatcgctg
551  tgctggtagg cagcttcgtc gcgaacaggt tctaccagag cggccacctg
601  gccgagtgcc tactggtcac gacgtatctg ctgatcgcaa ccgccgcagc
651  gctggccgtg ccgttgccgc gcggggtcaa ctcgttgggg gcgccacaag
701  ttgccggcgc cgctacggcc gtgctgtttt tgaccttgat gacgcggggc
751  ggccctcgga agcgtcatga gttggcgtcg tttgccgtga tcaccgctat
801  cgcggtcatc gcggccgccg ctgccttcgg ctatggatac caggactggg
851  tccccgcggg ggggatcgca ttcgggctgt tcattgtgac gaatgcggcc
901  aagctgaccg tcgcggtcgc gcggatcgcg ctgccgccga ttccggtacc
951  cggcgaaacc gtggacaacg aggagttgct cgatcccgtc gcgaccccgg
1001 aggctaccag cgaagaaacc ccgacctggc aggccatcat cgcgtcggtg
1051 cccgcgtccg cggtccggct caccgagcgc agcaaactgg ccaagcaact
1101 tctgatcgga tacgtcacgt cgggcaccct gattctggct gccggtgcca
1151 tcgcggtcgt ggtgcgcggg cacttctttg tacacagcct ggtggtcgcg
1201 ggtttgatca cgaccgtctg cggatttcgc tcgcggcttt acgccgagcg
1251 ctggtgtgcg tgggcgttgc tggcggcgac ggtcgcgatt ccgacgggtc
1301 tgacggccaa actcatcatc tggtacccgc actatgcctg gctgttgttg
```

FIG. 2 (continued)

```
1351 agcgtctacc tcacggtagc cctggttgcg ctcgtggtgg tcgggtcgat
1401 ggctcacgtc cggcgcgttt caccggtcgt aaaacgaact ctggaattga
1451 tcgacggcgc catgatcgct gccatcattc ccatgctgct gtggatcacc
1501 ggggtgtacg acacggtccg caatatccgg ttctga
```

mtbn7

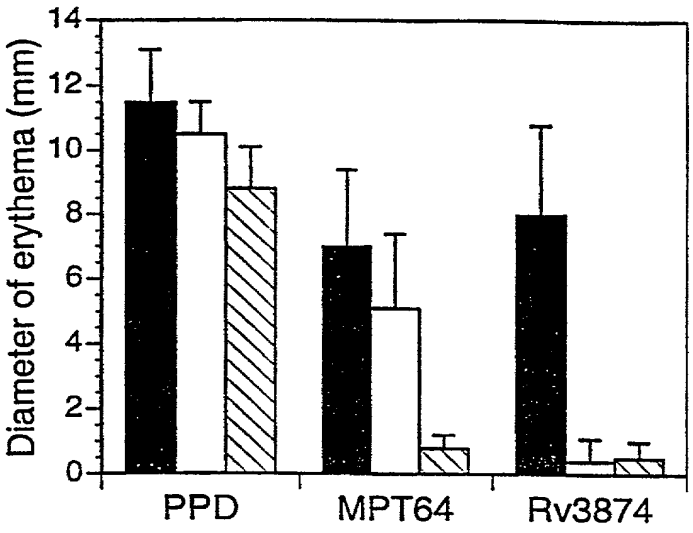
```
1    atggctgaac cgttggccgt cgatcccacc ggcttgagcg cagcggccgc
51   gaaattggcc ggcctcgttt ttccgcagcc tccggcgccg atcgcggtca
101  gcggaacgga ttcggtggta gcagcaatca acgagaccat gccaagcatc
151  gaatcgctgg tcagtgacgg gctgcccggc gtgaaagccg ccctgactcg
201  aacagcatcc aacatgaacg cggcggcgga cgtctatgcg aagaccgatc
251  agtcactggg aaccagtttg agccagtatg cattcggctc gtcgggcgaa
301  ggcctggctg gcgtcgcctc ggtcggtggt cagccaagtc aggctaccca
351  gctgctgagc acacccgtgt cacaggtcac gacccagctc ggcgagacgg
401  ccgctgagct ggcaccccgt gttgttgcga cggtgccgca actcgttcag
451  ctggctccgc acgccgttca gatgtcgcaa aacgcatccc ccatcgctca
501  gacgatcagt caaaccgccc aacaggccgc ccagagcgcg cagggcggca
551  gcggcccaat gcccgcacag cttgccagcg ctgaaaaacc ggccaccgag
601  caagcggagc cggtccacga agtgacaaac gacgatcagg gcgaccaggg
651  cgacgtgcag ccggccgagg tcgttgccgc ggcacgtgac gaaggcgccg
701  gcgcatcacc gggccagcag cccggcgggg gcgttcccgc gcaagccatg
751  gataccggag ccggtgcccg cccagcggcg agtccgctgg cggcccccgt
801  cgatccgtcg actccggcac cctcaacaac cacaacgttg tag
```

mtbn8

```
1    atgagtatta ccaggccgac gggcagctat gccagacaga tgctggatcc
51   gggcggctgg gtggaagccg atgaagacac tttctatgac cgggcccagg
101  aatatagcca ggttttgcaa agggtcaccg atgtattgga cacctgccgc
151  cagcagaaag gccacgtctt cgaaggcggc ctatggtccg gcggcgccgc
201  caatgctgcc aacggcgccc tgggtgcaaa catcaatcaa ttgatgacgc
251  tgcaggatta tctcgccacg gtgattacct ggcacaggca tattgccggg
301  ttgattgagc aagctaaatc cgatatcggc aataatgtgg atggcgctca
351  acgggagatc gatatcctgg agaatgaccc tagcctggat gctgatgagc
401  gccataccgc catcaattca ttggtcacgg cgacgcatgg ggccaatgtc
451  agtctggtcg ccgagaccgc tgagcgggtg ctggaatcca agaattggaa
501  acctccgaag aacgcactcg aggatttgct tcagcagaag tcgccgccac
551  ccccagacgt gcctaccctg gtcgtgccat ccccgggcac accgggcaca
601  ccgggaaccc cgatcacccc gggaaccccg atcaccccgg gaaccccaat
651  cacacccatc ccgggagcgc cggtaactcc gatcacacca acgcccggca
701  ctcccgtcac gccggtgacc ccgggcaagc cggtcacccc ggtgaccccg
751  gtcaaaccgg gcacaccagg cgagccaacc ccgatcacgc cggtcacccc
801  cccggtcgcc ccggccacac cggcaacccc ggccacgccc gttaccccag
851  ctcccgctcc acacccgcag ccggctccgg caccggcgcc atcgcctggg
901  ccccagccgg ttacaccggc cactcccggt ccgtctggtc cagcaacacc
951  gggcacccca gggggcgagc cggcgccgca cgtcaaaccc gcggcgttgg
1001 cggagcaacc tggtgtgccg ggccagcatg cgggcggggg gacgcagtcg
1051 gggcctgccc atgcggacga atccgccgcg tcggtgacgc cggctgcggc
1101 gtccggtgtc ccgggcgcac gggcggcggc cgccgcgccg agcggtaccg
1151 ccgtgggagc gggcgcgcgt tcgagcgtgg gtacggccgc ggcctcgggc
1201 gcggggtcgc atgctgccac tgggcgggcg ccggtggcta cctcggacaa
```

FIG. 2 (continued)

```
1251 ggcggcggca ccgagcacgc gggcggcctc ggcgcggacg gcacctcctg
1301 cccgcccgcc gtcgaccgat cacatcgaca aacccgatcg cagcgagtct
1351 gcagatgacg gtacgccggt gtcgatgatc ccggtgtcgg cggctcgggc
1401 ggcacgcgac gccgccactg cagctgccag cgcccgccag cgtggccgcg
1451 gtgatgcgct gcggttggcg cgacgcatcg cggcggcgct caacgcgtcc
1501 gacaacaacg cgggcgacta cgggttcttc tggatcaccg cggtgaccac
1551 cgacggttcc atcgtcgtgg ccaacagcta tgggctggcc tacatacccg
1601 acgggatgga attgccgaat aaggtgtact tggccagcgc ggatcacgca
1651 atcccggttg acgaaattgc acgctgtgcc acctacccgg ttttggccgt
1701 gcaagcctgg gcggctttcc acgacatgac gctgcgggcg gtgatcggta
1751 ccgcggagca gttggccagt tcggatcccg gtgtggccaa gattgtgctg
1801 gagccagatg acattccgga gagcggcaaa atgacgggcc ggtcgcggct
1851 ggaggtcgtc gacccctcgg cggcggctca gctggccgac actaccgatc
1901 agcgtttgct cgacttgttg ccgccggcgc cggtggatgt caatccaccg
1951 ggcgatgagc ggcacatgct gtggttcgag ctgatgaagc ccatgaccag
2001 caccgctacc ggccgcgagg ccgctcatct gcgggcgttc cgggcctacg
2051 ctgcccactc acaggagatt gccctgcacc aagcgcacac tgcgactgac
2101 gcggccgtcc agcgtgtggc cgtcgcggac tggctgtact ggcaatacgt
2151 caccgggttg ctcgaccggg ccctggccgc cgcatgctga
```


Diameter of erythema (mm)
14
12
10
8
6
4
2
0
PPD
MPT64
Rv3874

Attorney's Docket No.: 07763-043001
Client's Ref. No.:

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled PROTEINS EXPRESSED BY MYCOBACTERIUM TUBERCULOSIS AND NOT BY BCG AND THEIR USE AS DIAGNOSTIC REAGENTS AND VACCINES, the specification of which:

[] is attached hereto.
[] was filed on _ as Application Serial No. _ and was amended on _____.
[x] was described and claimed in PCT International Application No. PCT/US00/12257 filed on May 4, 2000 and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1 56.

I hereby claim the benefit under Title 35, United States Code, §119(e)(1) of any United States provisional application(s) listed below:

U.S. Serial No.	Filing Date	Status
60/132,505	May 4, 1999	Pending

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Combined Declaration and Power of Attorney

1 of 2

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